AWARD NUMBER: W81XWH-14-1-0271

TITLE: TARGETING GPR30 IN ABIRATERONE – AND MDV3100-RESISTANT PROSTATE CANCER

PRINCIPAL INVESTIGATOR: Hung-Ming Lam, PhD

CONTRACTING ORGANIZATION: University of Washington
Seattle, WA 98195

REPORT DATE: October 2015

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual report	30 Sep 2014 - 29 Sep 2015

4. TITLE AND SUBTITLE

TARGETING GPR30 IN ABIRATERONE – AND MDV3100-RESISTANT PROSTATE CANCER

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0271

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Hung-Ming Lam

E-Mail: minglam@uw.edu

5d. PROJECT NUMBER
0010505049

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Washington
1959 NE Pacific St
Seattle WA98195

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

New treatments to abiraterone (Abi)- and MDV3100 (MDV)-resistant prostate cancer have not been explored. G protein-coupled receptor 30 (GPR30) is a seven-transmembrane estrogen receptor and the activation by its specific agonist G-1 inhibited growth in multiple castration-resistant prostate cancer (CRPC) xenograft models that were resistant to the first-generation androgen deprivation therapy (ADT). More importantly, GPR30 is an androgen-repressed target and its expression increased in clinical CRPC when compared to primary prostate cancer. In this proposal, we will conduct preclinical studies to test the efficacy of G-1 in inhibiting the growth of prostate cancer that are resistant to the new second-generation ADT including Abi and MDV. We characterized two patient-derived xenograft models that are resistant to Abi and MDV, and the efficacy study of G-1 in inhibiting tumor growth are undergoing. We are also collecting clinical Abi- and MDV-resistant prostate cancer specimens from both biopsy and rapid autopsy to evaluate the prevalence of GPR30 expression in these advanced patients for potential targeting. This study will also provide information on the mechanism underlying GPR30 responsiveness and resistance.

15. SUBJECT TERMS
Prostate Cancer, Abiraterone, MDV3100, GPR30, Estrogen receptor, G-1, Patient derived xenografts, Treatment resistance

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
			Unclassified	8	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

1. ABSTRACT

Targeting GPR30 in Abiraterone- and MDV3100-Resistant Prostate Cancer

Lam H.M., Nguyen H.M., Olson J., Corey E.

Department of Urology, University of Washington, 1959 Pacific Street, Seattle, WA 98195

Period 9/30/14-9/29/15

New treatments to abiraterone (Abi)- and MDV3100 (MDV)-resistant prostate cancer have not been explored. G protein-coupled receptor 30 (GPR30) is a seven-transmembrane estrogen receptor and the activation by its specific agonist G-1 inhibited growth in multiple castration-resistant prostate cancer (CRPC) xenograft models that were resistant to the first-generation androgen deprivation therapy (ADT). More importantly, GPR30 is an androgen-repressed target and its expression increased in clinical CRPC when compared to primary prostate cancer. In this proposal, we will conduct preclinical studies to test the efficacy of G-1 in inhibiting the growth of prostate cancer that are resistant to the new second-generation ADT including Abi and MDV. We characterized two patient-derived xenograft models that are resistant to Abi and MDV, and the efficacy study of G-1 in inhibiting tumor growth are undergoing. We are also collecting clinical Abi- and MDV-resistant prostate cancer specimens from both biopsy and rapid autopsy to evaluate the prevalence of GPR30 expression in these advanced patients for potential targeting. This study will also provide information on the mechanism underlying GPR30 responsiveness and resistance.

2. INTRODUCTION

Castration-resistant prostate cancer (CRPC) is evolving fast and developing resistance to the most recent treatments including abiraterone (Abi) and MDV3100 (MDV). Treatments to these newly resistant tumors have not been explored. While research efforts continue to abolish the residue androgen signaling in these resistant cells, we propose to focus on androgen-repressed therapeutic targets whose expression is now high under the ultra-low androgen milieu in Abi- and MDV-resistant cancer. G protein-coupled receptor 30 (GPR30) is a seven-transmembrane estrogen receptor and it elicits cell growth or death depending on the cellular context. We showed GPR30 activation by its specific agonist G-1 inhibited prostate cancer growth through G2 arrest and apoptosis. We further showed that GPR30 expression was suppressed by androgen and importantly its expression was increased in castration-resistant prostate cancer (CRPC) in both preclinical setting and clinical specimens. G-1 inhibited the growth of multiple CRPC xenografts that were resistant to the first-generation ADT (i.e. castration). We hypothesize that for CRPC resistant to the second-generation ADT including Abi and MDV, the expression of the androgen-suppressed target GPR30 is high, and hence the anti-tumor effect of G-1 will be maximized.

In this proposal, we will perform preclinical testing on the efficacy and the safety of the GPR30-targeted therapy in our newly developed Abi- and MDV-resistant patient-derived xenografts, and investigate the frequency of GPR30 expression in patient specimens. This study will also provide information on the mechanism underlying GPR30 responsiveness and resistance.

3. KEYWORDS

Prostate Cancer, Abiraterone, MDV3100, GPR30, Estrogen receptor, G-1, Patient derived xenografts, Treatment resistance

4. ACCOMPLISHMENTS

In Year 1, we obtained approvals from IACUC and DoD ACURO in Feb 2015 for xenograft studies to investigate the efficacy of G-1-induced CRPC growth inhibition in abiraterone (Abi)- and MDV3100 (MDV)-resistant LuCaP xenografts. Since then, we have inoculated LuCaP 35CR and LuCaP 86.2 into male SCID mice and the tumors took more than expected (take rate of 78% and 83% for LuCaP 35CR and LuCaP 86.2, respectively). The mice were treated with Abi or MDV and resistance to drugs developed as anticipated **(Figure 1).** The mice were currently enrolled to G-1 treatment group upon Abi or MDV resistance at ~300mg on a rolling basis. No weight loss due to treatment was detected so far **(Figure 2)**. Tumor characterization and gene expression studies will be performed after the preclinical studies were finished.

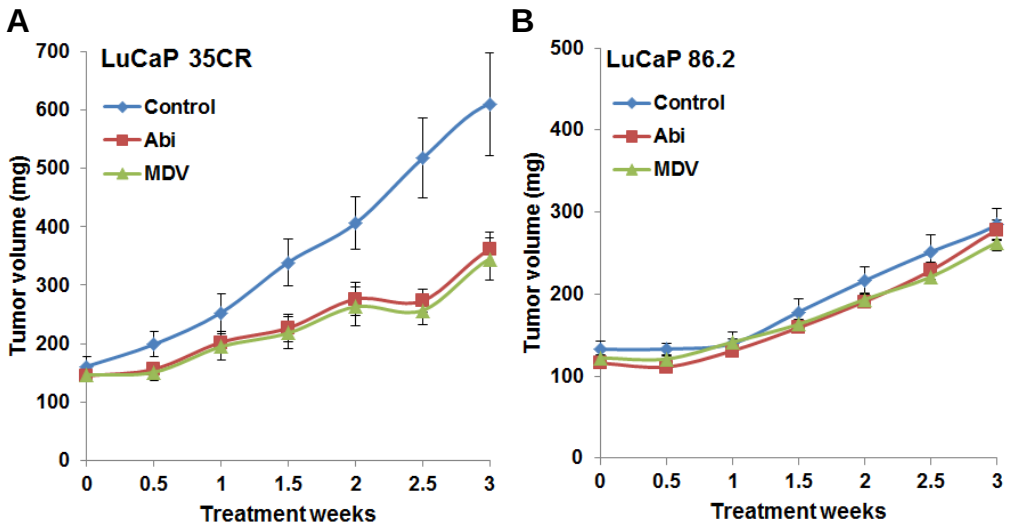

Figure 1. Tumor growth upon Abi and MDV resistance in **A)** LuCaP 35CR and **B)** LuCaP 86.2 patient-derived prostate cancer xenografts. Control, n=9; Abi, n=29-40 due to the rolling enrollment; MDV, n=21-37 due to the rolling enrollment.

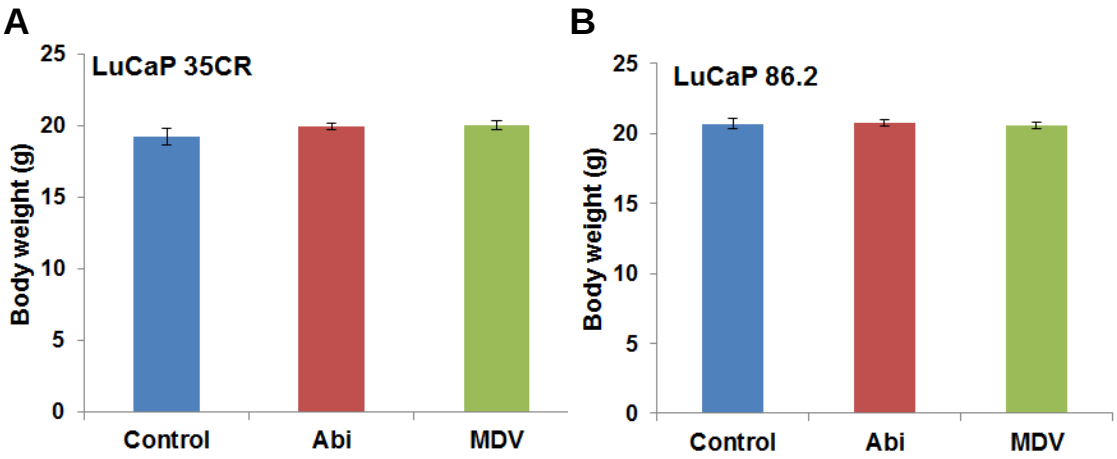

Figure 2. Body weight upon Abi and MDV resistance in **A)** LuCaP 35CR and **B)** LuCaP 86.2 patient-derived prostate cancer xenografts. Control, n=9; Abi, n=29-40 due to the rolling enrollment; MDV, n=21-37 due to the rolling enrollment.

In the past year with collaboration with Dr. Bruce Montgomery at the University of Washington we collected post-abiraterone biopsies from 15 patients (out of 30 patients over 3 years proposed). We also performed 10 rapid autopsies (out of 12 patients over 3 years proposed) and 8/10 patients were resistant to abiraterone and

7/10 patients were resistant to enzalutamide. We will send the tissues from rapid autopsies for intratumoral androgen analysis and perform the immunohistochemistry staining of GPR30 in Year 2 to reduce batch effects.

Opportunities for training and professional development
I participated in preclinical study meetings to gain knowledge about the different responses to abiraterone and MDV3100 on various in-house patient-derived xenograft models. The information will provide a basis for examining drug resistance outlined in this project.

Results disseminated to community of interest
1. Prepared a brief description of the project to 2015 PCRP program materials.
2. Presented part of the proposal in the planetary lecture in the Prostate Cancer Foundation Annual Retreat in October 2015.

Plan to do during the next reporting period to accomplish the goals
Since the experiments are going on track, we will adhere to the SOW and perform experiments as proposed. If unexpected issues occur, we will revise aims for approval accordingly.

5. IMPACT
Impact on the development of the principal discipline of the project
Nothing to report

Impact on other disciplines
Nothing to report

Impact on technology transfer
Nothing to report

Impact on society beyond science and technology
Nothing to report

6. CHANGES/PROBLEMS
Nothing to report

7. PRODUCTS
Publications, conference papers, and presentations
Targeting estrogen receptors in castration-resistant prostate cancer, lecture at Prostate Cancer Foundation 22nd Scientific Retreat, Washington DC. October 2015.

8. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Name	Hung-Ming Lam
Project role	PD/PI
Nearest person month worked	2
Contribution to project	Prepare documents for IACUC, ACURO, and HRPO approvals, and IRB exemptions; design and oversee preclinical studies; analyzing results; clear documents required for abiraterone acetate and MDV3100 transfer

Name	Jessica Olson
Project role	Research scientist
Nearest person month worked	2

Contribution to project	Perform preclinical studies including castration of the mouse, tumor inoculation, tumor measurement, drug administration, PSA measurement, mouse sacrifice, and tissue acquisition; acquire abiraterone- and MDV3100-resistant specimens in the prostate cancer rapid autopsy program

Name	Holly Nguyen
Project role	Research scientist
Nearest person month worked	2
Contribution to project	Submit IACUC protocol; perform preclinical studies and organize results; acquire abiraterone- and MDV3100-resistant specimens in the prostate cancer rapid autopsy program
Funding support	NIH/NCI

Changes in active support
Nothing to report

Other organizations involved as partners

Organization name	Janssen Pharmaceuticals
Location of organization	Raritan, NJ
Contribution to the project	Provided abiraterone acetate for the study

Organization name	Astella/Medivation
Location of organization	San Francisco, CA
Contribution to the project	Provided MDV3100 for the study

9. SPECIAL REPORTING REQUIREMENTS
N/A

10. APPENDICES
N/A